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Analysis of 3-D Ultrasound of Calf Muscle Geometry in Children: Growth, Spasticity, Mechanisms and Treatment

Children with spastic cerebral palsy (SCP) are often impaired in their gait as a result of a decreased range of motion (ROM) of their ankle joint. One of the most common treatments of the decreased ROM is injection of botulinum toxin A (BTX-A) into the spastic ankle plantar flexor muscles. The rationale behind this treatment is to temporarily decrease activity of the spastic muscles (by blocking motor endplates), therefore allowing an increase in ankle ROM. BTX-A treatment is usually followed by a period of serial casting of the ankle towards dorsal flexion to maintain an extended position of the plantar flexor muscles in an effort to stimulate longitudinal growth of the muscle fascicles and hence an increase in ankle dorsal flexion ROM. In the short term, ankle dorsal flexion is increased after such treatment. However, in the long-term, success of this integrated treatment is highly variable. To date, little is known about the mechanisms by which BTX-A and serial casting affect the ankle plantar flexor muscles in children with SCP. Such knowledge is required for improvement of the efficacy of treatment.

The general aim of the studies of which this thesis is a part, is to investigate how ankle plantar flexor muscles are changed in children with SCP compared to typically developing (TD) peers and to investigate how such changes are affected by the integrated treatment of BTX-A and serial casting.

For this purpose, ultrasound imaging of the medial gastrocnemius (GM) muscle and dynamometry of the ankle were performed. Measurements of muscle geometry were performed in a standardized fascicle plane of GM using three-dimensional (3-D) ultrasound imaging. First, in **Chapter 2**, we validated two-dimensional (2-D) ultrasound measurements of muscle geometry of the human GM and investigated effects of probe orientation on errors in these measurements. Ultrasound scans of GM muscle belly were made both on human cadavers and on subjects *in vivo*. For half of the cadavers, ultrasound scans obtained, according to commonly applied criteria of probe orientation, showed substantial deviation from the true fascicle plane. This resulted in errors of fascicle length and fascicle angle up to 14% and 23%, respectively. Fascicle-like structures not visibly distinguishable from true fascicles were seen over a wide range of probe

tilt and rotation angles, but they did not always represent true fascicles. Errors of measurement were either linear or quadratic functions of tilt angle. As similar curves were found also *in vivo* we conclude that such errors are likely to occur for *in vivo* measurements. For all cadavers, at the distal end of GM, the true fascicle plane was shown to be perpendicular to the distal aponeurosis. Using transverse images of GM to detect the curvature of the distal aponeurosis at the distal end of the muscle belly is a simple strategy to help identify the fascicle plane. For subsequent longitudinal imaging, alignment of the probe within this plane will help to minimize measurement errors of fascicle length, fascicle angle, and muscle thickness.

In order to measure static ankle angle and moment in children with cerebral palsy, while correcting for aspects of foot deformity, we designed a hand-held instrument (described in Chapter 3) which can be used to measure the angle of the footplate (as an estimate of the ankle angle) and the moment applied at the footplate.

In **Chapter 3**, the reproducibility of measurements performed with this instrument was tested. Footplate angles and moments were measured at five standardized positions in TD children (n=10), as well as in children with SCP (n=10). The footplate range of motion and the slope of the moment-angle curve were determined, both towards plantar flexion and dorsal flexion. The intraclass correlation coefficients for angle and moment were calculated to assess test-retest reliability. For precision, the standard error of measurement and smallest detectable difference for angle and moment measurement were determined. It was concluded that our instrument allows for reliable and precise measurements of footplate angle and static moment in children with SCP and TD peers. The hand-held dynamometer is fit for reproducibly evaluating moment-angle characteristics in development and clinical contexts.

To understand how spastic GM develops, in the study presented in **Chapter 4**, we studied the development of the GM of TD children. During development, muscle growth is thought to be adapted finely to meet functional demands in daily activities. Most knowledge of how muscles grow is based on animal studies and in particular rodents. Using 3-D ultrasound imaging and a measurement protocol based on Chapter 2 and 3, GM geometry of the mid-longitudinal plane of GM was selected and

analyzed using a 3-D voxel array of ultrasound images. These images were obtained at standardized footplate angles according to added external moments using the hand-held dynamometer. GM geometry and footplate ROM of TD children, specifically in the age of five to thirteen years, were examined. GM geometry and length variables of TD children were compared to those of rats. This comparison showed that GM in rat develops mainly by an increase in physiological cross-sectional area of the muscle, whereas GM in TD children develops by uniform scaling of the muscle, also including an increase in fascicle length. This effect is probably related to the lower degree of pennation (e.g. fascicle angle) in human GM, which causes a lower contribution of radial muscle fiber growth to increased GM muscle length. A net effect of uniform scaling of GM muscle belly during development is a stiffening of the muscle belly which is in accordance with the observed decrease in dorsal flexion ROM during growth.

A next step was to compare GM length and geometry of children with SCP to that of TD peers. To minimize the effects of growth on GM geometry shown in Chapter 4, in the study presented in **Chapter 5**, children in both groups were studied within a smaller age range (i.e. nine to thirteen years). It was found that in children with SCP, diagnosed with decreased dorsal ROM of the ankle, GM length was substantially lower compared to that in TD peers. After normalization for tibia length and measured at similar footplate angles, GM was about 1 cm shorter in children with SCP. This may be related to the lower malleolus height: such a lower malleolus height could be explained by a relative plantar flexed calcaneus, which an X-ray study performed on one child from the SCP group and one TD adult revealed. In addition, in children with SCP the moment-normalized fascicle length curve was substantially higher, indicating that GM fascicle stiffness was considerably increased. This increase in stiffness was explained partially by a lower fascicle length and thus indicates that factors other than myofiber length and stiffness are involved in the enhanced GM stiffness. It seems conceivable that intracellular material properties of muscle fibers, intramuscular connective tissue and/or epimuscular myofascial force transmission are altered such that resistance to lengthening is increased. As yet, the relative contribution of these factors to the enhanced stiffness is unknown. However these factors are

important to consider for improvement of treatment aimed at increasing ankle ROM in children with a spastic pareses.

One of the most commonly used treatments modalities aimed at increasing ankle ROM is BTX-A and serial casting. The questions whether this integrated treatment increases the ROM and whether such an increase is related to changes in GM geometry are addressed in **Chapter 6**. This is done for short-term (<5 weeks) effects of integrated treatment of BTX-A and serial casting of the lower leg. Length and geometric variables of GM related to footplate ROM were assessed in six children with SCP (age: 9-13y) treated with BTX-A and serial casting of the lower leg. The children were tested pre- and post treatment. This study shows that a short-term gain of dorsal ROM in children with SCP after treatment was ascribable to an increase in GM fascicle length, as well as GM tendon length. Within the short-term, the treatment did not induce atrophy of GM in the mid-longitudinal plane.

This is first step in analyzing our collected data on effects of BTX-A and serial casting on GM geometry and reduced ROM in children with SCP. A detailed analysis whether the achieved improvements and changes in muscle geometry remain on the long-term needs further investigation. How the treatment changes the foot deformity and whether the effects are similar during development are important questions which need further analysis of the data. Our future studies on these data will focus on determinants such as time-scale (short vs. long-term effects).

Finally, in **Chapter 7** the results of all studies are considered in perspective of the overall aim of this project. First, some methodological considerations concerning ultrasound imaging analysis and hand-held dynamometry are discussed.

Next, some future directions and clinical implications of the work of this thesis are discussed. These issues concern growth related changes of GM geometry in children with SCP, development of altered ankle and foot joints in children with SCP, long-term effects of BTX-A and serial casting, differences in children and rodents concerning effects of immobilization of GM at high length and effects of age on treatment. To understand how GM of children with SCP is affected by spasticity and how this is changed in the long-term after the integrated treatment of BTX-A and serial casting,

X-ray imaging of the foot and long-term *in vivo* measurements after treatments are required.

In addition, biopsy studies of GM fibers may enhance our understanding of how muscle and joints are affected by spasticity and how this changes in the long-term by integrated treatment of BTX-A and serial casting. Such studies may also contribute to our understanding of expected changes in myofascial force transmission of spastic muscles in children with SCP.

In conclusion, the work presented in this thesis shows that the combined analysis of muscle geometry using 3-D ultrasound imaging and ankle dynamometry provides valuable insight in the mechanisms underlying a limited ROM of the ankle of children with SCP. Compared to TD peers, substantial differences in GM fascicle length of children with SCP are present at similar applied external moments. The difference in fascicle length contributes to the lower dorsal ROM of the ankle.

After treatment with BTX-A and serial casting, dorsal ROM was increased by an increase in GM fascicle length and tendon length. Foot deformities likely play an important role in the etiology of the decreased ROM.

Biopsy studies of GM fibers and X-ray imaging of the foot, in addition to long-term *in vivo* measurements after treatment are required to further understand how GM of children with SCP is affected by spasticity and how this is affected in the long-term after integrated treatment of BTX-A injection and serial casting.